

# Assessment of Experimental Bt Events Against Fall Armyworm and Corn Earworm in Field Corn

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**ABSTRACT** Performance of experimental *Bacillus thuringiensis* (Bt) MON events alone and pyramided with MON810 were evaluated over 3 yr in Georgia and Alabama. Ability of events to prevent whorl defoliation by the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and natural ear feeding damage by the corn earworm, *Helicoverpa zea* (Boddie) was assessed. In each year, near-isogenic hybrids with novel single transformation events and crosses pyramided with the MON810 event were compared with the standard single MON810 event and nontransformed susceptible control. Events were tested for resistance to whorl damage by manual infestations of fall armyworm and ear damage by natural infestations of corn earworm. All Bt events tested reduced fall armyworm whorl damage ratings per plant compared with the susceptible hybrid. All Bt treatments also had considerably less ear infestation and damage by corn earworm compared with the nontransgenic isolate. The MON841, MON849, and MON851 events reduced ear damage by *H. zea* but were not as effective as other novel events and were not advanced for further testing after the 1999 season. Pyramiding events compared with single events did not improve control of fall armyworm whorl damage, but they generally did prevent more ear damage by corn earworm. The MON84006 event singly and pyramided with MON810 had superior control of whorl-stage damage by *S. frugiperda* and ear damage by *H. zea* compared with MON810. Deployment of new events and genes could provide additional tools for managing the potential for insect resistance to Bt toxins. Furthermore, improved control of whorl and ear infestations by *H. zea* and *S. frugiperda* would increase the flexibility of planting corn, *Zea mays* L., and permit double cropping of corn in areas where these pests perennially reach damaging levels.

**KEY WORDS** corn earworm, fall armyworm, Bt corn, biopesticides, maize

TRANSGENIC CORN, *Zea mays* L., HYBRIDS EXPRESSING the Cry insecticidal protein from *Bacillus thuringiensis* (Bt) were initially developed to control European corn borer, *Ostrinia nubilalis* (Hübner), and southwestern corn borer, *Diatraea grandiosella* Dyar (Ostlie et al. 1997). Several events of transgenic Bt corn have been developed with different modes of toxin expression (Ostlie et al. 1997). In 2002, the only commercially deployed Bt resistance in corn in the southeastern United States was the Cry1Ab gene as events MON810 (Monsanto Co., St. Louis, MO) and Bt11 (Syngenta Seeds, Raleigh, NC). Hybrids containing either of these events are collectively referred to as having “YieldGard Corn Borer Technology” (Ostlie et al. 1997). Endotoxin in these events is expressed in vegetative and reproductive structures throughout the season (Armstrong et al. 1995, Williams et al. 1997).

The benefits of Bt-resistant corn largely depend on the extent and severity of target pest infestations (EPA

2001). YieldGard Corn Borer resistance is very effective against European and southwestern corn borers (Williams et al. 1998, Archer et al. 2000), but these insects either do not occur or are not economically important in most of the coastal plain region of the southeastern United States. Bt resistance offers the potential for reducing damage by fall armyworm, *Spodoptera frugiperda* (J.E. Smith) and corn earworm, *Helicoverpa zea* (Boddie), which are the key lepidopteran pests of corn in this area. Laboratory feeding trials and small controlled field trials have shown that hybrids containing the Bt11 event reduce fall armyworm and corn earworm growth and survival (Williams et al. 1997, 1998). In a series of field trials at several locations in Georgia and Alabama in 1998 with corn planted at the recommended time and 1 and 2 mo later, hybrids with MON810 or Bt11 events prevented whorl damage, kernel damage, and yield loss by lepidopterans, primarily fall armyworm and corn earworm, in later plantings at all locations (DeLamar et al. 1999a,b,c,d,e; Buntin et al. 2001). Bt hybrids in these trials reduced whorl damage by >90% but only reduced ear damage by 50–70%. Bt hybrids generally did not improve the yield performance of corn planted at recommended times, because corn planted at these times usually avoided severe lepidopteran damage in

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Table 1. Effect of Bt events on fall armyworm whorl damage ratings<sup>a</sup> ( $\pm$  SEM) in three trials in Georgia and Alabama in 1999

Event	Griffin		Tifton		Alabama	
	9 d <sup>b</sup>	16 d	7 d	14 d	7 d	14 d
Non Bt Control	2.85 $\pm$ 0.22a	2.99 $\pm$ 0.28a	3.31 $\pm$ 0.08a	2.16 $\pm$ 0.08a	1.36 $\pm$ 0.38a	1.60 $\pm$ 0.49a
MON 810	0.13 $\pm$ 0.66b	0.07 $\pm$ 0.02b	1.30 $\pm$ 0.03b	1.42 $\pm$ 0.05bc	0.20 $\pm$ 0.09b	0.27 $\pm$ 0.18b
MON 849	0.20 $\pm$ 0.07b	0.17 $\pm$ 0.06b	1.39 $\pm$ 0.05b	1.21 $\pm$ 0.05c	0.32 $\pm$ 0.12b	0.40 $\pm$ 0.12b
MON 851	0.26 $\pm$ 0.09b	0.12 $\pm$ 0.02b	1.64 $\pm$ 0.04b	1.77 $\pm$ 0.07ab	0.29 $\pm$ 0.12b	0.32 $\pm$ 0.21b
MON 840	0.03 $\pm$ 0.02b	0.03 $\pm$ 0.03b	0.16 $\pm$ 0.04c	0.28 $\pm$ 0.05d	0b	0.15 $\pm$ 0.07b
MON 840/MON 810	0.09 $\pm$ 0.03b	0.01 $\pm$ 0.01b	0.12 $\pm$ 0.03c	0.06 $\pm$ 0.02d	0b	0.26 $\pm$ 0.09b
MON 841	0.03 $\pm$ 0.02b	0.03 $\pm$ 0.02b	0.15 $\pm$ 0.03c	0.20 $\pm$ 0.03d	0.03 $\pm$ 0.03b	0.07 $\pm$ 0.05b
MON 841/MON 810	0.03 $\pm$ 0.02b	0.01 $\pm$ 0.01b	0.11 $\pm$ 0.03c	0.06 $\pm$ 0.02d	0.08 $\pm$ 0.08b	0.33 $\pm$ 0.09b

Means within columns followed by the same letter are not significantly different (protected LSD,  $P < 0.05$ ).

<sup>a</sup> Whorl damage rating scale (1–9) 1 = no damage, 9 = whorl and furl mostly destroyed of (Davis et al. 1992).

<sup>b</sup> days after infestation at 7–9-leaf stage.

most years. However, Bt hybrids prevented yield losses that were  $>50\%$  in some cases in late plantings. In North Carolina, corn containing Bt11 or MON810 events reduced ear damage by *H. zea* by 80% and reduced *H. zea* emergence from Bt cornfields by  $\approx 75\%$  (Storer et al. 2001). Furthermore, Bt hybrids also stunted the growth of surviving *H. zea* larvae and delayed adult emergence by 6–12 d.

Insect resistance management for Bt plant-incorporated biopesticides consists of a high-dose/refuge strategy. High dose is defined as 25 times the protein concentration necessary to kill susceptible larvae (Gould 1994). Neither YieldGard event is considered to have a high-dose effect on *H. zea* or *S. frugiperda* (EPA 2001). New Bt events and genes singly or pyramided (i.e., multiple toxins, expressed simultaneously in the same hybrid) with the MON810 event may increase the dose response to these species and improve control of these pests in corn in the southern United States. If effective, new genes could provide additional tools for managing the potential for insect resistance to Bt toxins, thereby reducing the exposure of *H. zea* populations the Cry1A toxins currently used in both Bt corn and Bt cotton. This study examines the field performance of novel Bt MON events alone and pyramided with MON810 in preventing whorl defoliation by the fall armyworm and natural ear feeding damage by the corn earworm in Georgia and Alabama.

### Materials and Methods

Trials in 1999 were conducted at the University of Georgia Dempsey Research Farm, near Griffin, GA, the Coastal Plain Experiment Station near Tifton, GA, and at the Alabama Agricultural Experiment Station E. V. Smith Research Center near Tallahassee, AL. Second and third trials were conducted at Griffin, GA, and Tallahassee in 2000 and 2001. Conventional tillage was used for all trials. Fertilizer was broadcasted and incorporated before planting, and nitrogen was applied as a side-dress application  $\approx 20$  d after planting. Seed was planted from 10 to 23 May in each year at the rate of 4.9 seed per meter of row. Row spacing was 76 cm in all trials except at Tifton in 1999, where rows were 91 cm apart. Plots were two rows by 6.1 m with

two border rows of a hybrid containing MON810 between plots. In the 1999 Tifton trial and 2001 Griffin trial, plots were four rows wide with no border rows. All seed was treated with a commercial fungicide. No insecticide or foliar fungicides were applied. Weeds were controlled using preemergence application of atrazine at 2.1 kg AI/ha (Aatrex 4L, Syngenta, Greensboro, NC) or atrazine plus pendamethalin at 0.84 kg AI/ha (Prowl 3.3 EC, BASF Corp., Research Triangle Park, NC).

Plots were arranged in a randomized complete block design with four to six replications per trial. In each year, near-isogenic hybrids with novel single transformation events and crosses with the MON810 event were compared with the standard single MON810 event and nontransformed susceptible control. Novel events in 1999 were MON840, MON841, and MON849, and MON851. MON840, and MON841 pyramided with MON810 also were included. Events in 2000 were MON84005 and MON84006 singly and pyramided with MON810. MON849 and MON851 events contained the Cry1Ab gene. MON840, MON841, MON84005, and MON84006 contained the Cry2Ab gene. The 2001 trial evaluated the MON84006 event singly and pyramided with MON810.

Hybrids were evaluated for whorl-stage fall armyworm efficacy. Plants were manually infested at about the seven-leaf stage of development with one application of 30 fall armyworm neonates per plant or two applications of 15 neonates per plant by using the bazooka method (Wiseman et al. 1980). In the 2001 Griffin trial, fall armyworm infestation was achieved by placing one fully developed (black-head stage) egg mass with 20–40 eggs per mass in the whorl of each plant. Fall armyworm eggs were provided by DeKalb Seeds/ Monsanto, except in the Tifton 1999 trial where eggs came from a colony at the USDA Insect Biology and Population Management Research Laboratory at Tifton. At 7 and 14 d after infestation, all plants were rated individually for leaf-feeding damage by using the 0–9 scale of Davis et al. (1992), where 0 is no damage and 9 is whorl and furl mostly destroyed.

Ear resistance to corn earworm feeding injury was evaluated in the 1999 Georgia trials by placing one application of  $\approx 40$  corn earworm eggs per ear or two

**Table 2.** Effect of Bt events on fall armyworm whorl damage rating<sup>a</sup> at 14 d after infestation of late whorl stage corn in Georgia and Alabama in 2000 and 2001

Event	2000		2001	
	GA	AL	GA	AL
Non-Bt Control	1.07 ± 0.09a	6.22 ± 0.06a	1.63 ± 0.44a	2.53 ± 0.19a
MON 810	0.05 ± 0.02b	2.10 ± 0.19b	0.06 ± 0.01b	0.85 ± 0.07b
MON 84005	0.03 ± 0.01b	0.90 ± 0.06c	—	—
MON 84005/MON 810	0.03 ± 0.2b	0.88 ± 0.08c	—	—
MON 84006	0.05 ± 0.02b	0.88 ± 0.18c	0.08 ± 0.04b	0.52 ± 0.14b
MON 84006/MON 810	0.05 ± 0.04b	1.13 ± 0.16c	0.01 ± 0.01b	0.65 ± 0.08b

Means within columns followed by the same letter are not significantly different (protected LSD,  $P < 0.05$ ).

<sup>a</sup> 0–9 whorl damage rating scale using 14-d scale (Davis et al. 1992).

applications of  $\approx 20$  eggs per ear. Eggs were applied at the base of emerged silks of each ear by using an agar-solution pressure applicator (Wiseman et al. 1974) or the bazooka technique. Corn earworms naturally infested plots in the 1999 Alabama trial and in all trials in 2000 and 2001. In 2000 trials, ears also were infested with 40 fall armyworm neonates by using the bazooka technique but very few armyworms established in any trial. At the milk stage,  $\approx 10$ –14 d after infestation in 1999, 10 or 20 ears per plot were sampled for number of larvae, larval instar, and rated for ear damage (Widstrom 1967). Ear damage also was measured on 10 or 20 additional ears at physiological maturity when larvae had finished feeding on all hybrids.

Results were analyzed by trial and sample date via analysis of variance (ANOVA) for a randomized complete block design using PROC GLM (SAS Institute 1999). Means were separated using Fisher's protected least significant difference (LSD) at  $P = 0.05$ . Percentage data were transformed with an arcsine square-root transformation before analysis.

## Results

**Fall Armyworm Whorl Infestation.** All Bt events in 1999 significantly reduced mean whorl damage rating per plant compared with the nontransgenic isolate on all sample dates (Table 1). However at Tifton, plants with the MON810, MON849, and MON851 events had more leaf-feeding damage than plants with MON840 and MON841 events singly and pyramided with

MON810. Otherwise, there were no significant differences in percentage of infested whorls or whorl damage ratings between hybrids containing Bt events on any sample date in the Griffin or Alabama trials. All events in the 2000 and 2001 trials significantly reduced mean whorl damage rating per plant compared with the nontransgenic isolate on all sample dates (Table 2). Leaf damage ratings were not different between Bt events except in Alabama in 2000 where all new events had less leaf damage than MON810.

**Ear Infestation and Damage.** Because of the late planting time, large natural infestations of corn earworms supplemented manual infestations, resulting high levels of ears infestation in all 1999 trials (Table 3). At Griffin, all Bt events except MON810 reduced the percentage of infested ears; however, the MON840 and MON840/MON810 events had significantly fewer infested ears than all other treatments. Likewise, events in all 1999 trials reduced ear damage ratings compared with the susceptible hybrids except MON810 at Tifton. Nevertheless, ear damage ratings of MON810, MON849, and MON841 were significantly greater than ear damage to the MON840 and the two pyramided events (Table 3). Ear damage to the MON851 event was variable between trials. The number of damaged kernels per ear in the Alabama trial was substantially lower in all Bt events than the susceptible hybrid with MON840, MON851, MON840/MON810, and MON841/MON810 having the fewest number of damaged kernels. Addition of

**Table 3.** Effect of Bt events on ear infestations and damage by corn earworm in Georgia and Alabama trials in 1999

Event	Infested ears (%) Griffin	Infested ears (%) Alabama	Ear damage rating <sup>a</sup>			Damaged kernels <sup>b</sup>
			Griffin	Tifton	Alabama	
Non-Bt Control	100.0 ± 0.0a	85.0 ± 12.9a	4.56 ± 0.13a	4.15 ± 0.26a	4.74 ± 0.45a	28.0 ± 3.6a
MON 810	95.0 ± 2.2ab	43.3 ± 4.3b	2.42 ± 0.39b	4.44 ± 0.26a	2.00 ± 0.15c	6.4 ± 0.9cd
MON 849	93.0 ± 3.4b	45.0 ± 4.2b	2.44 ± 0.13b	3.86 ± 0.25ab	2.05 ± 0.14c	7.9 ± 1.0c
MON 851	26.0 ± 5.8d	15.0 ± 3.2cd	0.38 ± 0.13c	3.08 ± 0.21bc	0.65 ± 0.22de	1.3 ± 0.3e
MON 840	6.0 ± 3.7e	1.7 ± 1.7e	0.26 ± 0.05e	0.47 ± 0.12d	0.05 ± 0.05e	0.2 ± 0.2e
MON 840/MON 810	4.0 ± 1.9e	5.0 ± 3.2de	0.05 ± 0.03c	0.32 ± 0.10d	0.45 ± 0.26de	0.8 ± 0.5e
MON 841	71.0 ± 5.3c	56.7 ± 3.3b	2.64 ± 0.21b	2.23 ± 0.24c	3.50 ± 0.45b	14.5 ± 2.3b
MON 841/MON 810	29.0 ± 8.3d	20.0 ± 4.7c	0.58 ± 0.19c	0.79 ± 0.17d	1.22 ± 0.31cd	2.0 ± 0.8de

Means within columns followed by the same letter are not significantly different (protected LSD,  $P < 0.05$ ). Percentage values transformed with arcsine square root transformation before analysis.

<sup>a</sup> Widstrom (1967).

<sup>b</sup> Alabama 1999 trial.

Table 4. Effect of Bt events on natural ear infestations and damage by corn earworm in Georgia and Alabama trials in 2000

Event	Infested Ears (%)		Larvae per ear		Damage rating per ear <sup>a</sup>	
	GA	AL	GA	AL	GA	AL
Non-Bt Control	97.5 ± 1.1a	83.3 ± 4.3a	1.09 ± 0.11a	0.93 ± 0.07b	5.40 ± 0.51a	6.15 ± 0.61a
MON 810	45.0 ± 6.8c	30.0 ± 11.4b	0.18 ± 0.04bc	0.27 ± 0.05c	0.18 ± 0.02b	2.30 ± 0.59c
MON 84005	84.2 ± 2.0b	75.0 ± 7.4a	1.00 ± 0.10a	1.21 ± 0.14a	3.99 ± 1.17a	3.87 ± 0.49b
MON 84005/MON 810	29.2 ± 5.5d	16.7 ± 4.3bc	0.24 ± 0.07b	0.13 ± 0.05c	0.30 ± 0.06b	1.13 ± 0.18cd
MON 84006	16.7 ± 4.4e	17.1 ± 6.1bc	0.12 ± 0.03bc	0.19 ± 0.08c	0.63 ± 0.18b	1.25 ± 0.36cd
MON 84006/MON 810	5.0 ± 1.3f	5.0 ± 3.2c	0.01 ± 0.01c	0.05 ± 0.03c	0.01 ± 0.01b	0.78 ± 0.25d

Means within columns followed by the same letter are not significantly different (protected LSD,  $P < 0.05$ ). Percentage values transformed with arcsine square root transformation before analysis.

<sup>a</sup> Widstrom (1967).

the MON810 event did not improve the efficacy of the MON840 event singly in preventing ear damage by *H. zea*. Conversely, addition of the MON810 event improved the efficacy of the MON841 compared with either single event (Table 3).

Ear infestations in 2000 and 2001 trials consisted almost entirely of natural infestations of corn earworm. Fall armyworms accounted for <5% of larvae in 2000, and no fall armyworms were found in the 2001 trials. All events reduced percentage of infested ears and ear damage rating in all trials except MON810 in the 2001 Alabama trial (Tables 4 and 5). The other exception was the MON84005 event, which did not effectively reduce ear infestation or damage in both 2000 trials. Pyramiding MON810 and MON84005 reduced ear damage compared with MON84005 but did not reduce damage compared with MON810 singly. MON84006 was the most effective single event. Addition of MON810 to MON84006 reduced damage in some cases compared with either single event.

All events except MON84005 also reduced the numbers of corn earworm larvae per ear (Tables 4 and 5). The MON84006 and MON84006/MON810 events had very few larvae present in all 2000 and 2001 trials. All events tended to delay larval development in 2001 as indicated by reduction in average instar of larvae in the ear ≈2 wk after green silk stage, but this trend was significant in both trials only for the MON84006 event (Table 5).

### Discussion

All Bt events reduced fall armyworm whorl damage ratings per plant compared with the susceptible hybrid. MON849 and MON851 were similar to the stan-

dard MON810 in preventing whorl damage, but all three events had more whorl damage at the Tifton location in 1999 than MON840 and MON841 singly and pyramided with MON810. However, pyramiding events did not reduce fall armyworm whorl damage compared with MON840 and MON841 single events.

Despite manual infestations of ears with *S. frugiperda* in 2000, virtually all lepidopterans recovered from corn ears were *H. zea*. All Bt treatments also had considerably less ear infestation and damage by corn earworm compared with the nontransgenic isolate. The MON841, MON849, MON851 also reduced ear damage by *H. zea* but were not as effective as the MON840 event. Consequently, these three events were not advanced for further testing after the 1999 season. Pyramiding MON840 and MON841 with MON810 improved the efficacy of both single events, but the improved performance was more pronounced with MON841 than MON840 and MON84006. The hybrid with the pyramided MON84006/MON810 events consistently had the lowest amount of whorl and ear infestation and damage in the 2000 and 2001 trials. Therefore, the pyramided treatment of MON 84006/MON810 provided superior ear protection compared with either event alone.

A high-dose expression causing 99% or more mortality of susceptible insects is an essential component of the high-dose/refuge strategy for reducing the risk of resistance to Bt toxins in target pests. Exposure of insect populations to Bt-incorporated plants with moderate dosage levels could greatly increase the likelihood of resistance developing in susceptible populations (Gould et al. 1995). The risk for resistance to Bt toxins by *H. zea* is greater in cotton-growing areas than noncotton areas, because of the potential for

Table 5. Effect of Bt events on natural ear infestation and damage by corn earworm in Georgia and Alabama trials in 2001

Event	Infested ears (%)		Live larvae per ear		Larval instar		Damage rating per ear <sup>a</sup>	
	GA	AL	GA	AL	GA	AL	GA	AL
Non-Bt Control	80.8 ± 5.7a	91.7 ± 1.7a	0.77 ± 0.17a	0.76 ± 0.07a	4.19 ± 0.10a	4.98 ± 0.02a	2.02 ± 0.20a	5.73 ± 0.79a
MON 810	33.3 ± 5.6b	86.7 ± 4.7a	0.28 ± 0.09bc	0.92 ± 0.19a	2.79 ± 0.10b	3.70 ± 0.14ab	0.59 ± 0.08b	4.83 ± 0.73ab
MON 84006	11.7 ± 2.5c	33.3 ± 4.7b	0.11 ± 0.04c	0.18 ± 0.05b	2.99 ± 0.21b	3.45 ± 0.75b	0.51 ± 0.13b	1.75 ± 0.14b
MON 84006/MON810	2.5 ± 1.7d	26.7 ± 5.4b	0c	0.18 ± 0.10b	—	4.31 ± 0.57a	0.09 ± 0.04c	2.10 ± 0.54b

Means within columns followed by the same letter are not significantly different ( $P < 0.05$ , protected LSD); percentage values transformed with arcsine square root transformation before analysis.

<sup>a</sup> Widstrom (1967).



repeated exposure of consecutive generations to cry toxins in Bt corn (Cry1Ab) and Bt cotton (Cry1Ac). Cotton-growing areas include the entire southeastern U.S. coastal plain region. To reduce the potential exposure of *H. zea* to cry toxins, the refuge requirement in cotton-growing areas is that Bt corn cannot be planted on >50% of a farm's corn acreage compared with 20% in noncotton-growing areas (EPA 2001). Further analysis is required to determine whether an improvement in efficacy against *H. zea* by MON84006 compared with MON810 would justify changes in the refuge requirement.

The MON810 and MON84006 events singly and pyramided exhibited very good levels of whorl stage control of *S. frugiperda* in all years. Ears infestations by *S. frugiperda* did not occur in this study but ear infestations by *S. frugiperda* can occur on hybrids with MON810 event (DeLamar et al. 1998a,b,c,d,e; Buntin et al. 2001). Thus, MON810 and probably MON84006 do not have a season-long high-dose expression for *S. frugiperda*. Whereas *S. frugiperda* has multiple generations with numerous host plants, it is primarily a grass feeder and is not a major pest of cotton (Sparks 1979). Therefore, at present, the potential for repeated exposure of *S. frugiperda* populations to endotoxins in Bt corn and Bt cotton during a season seems minimal. Furthermore, *S. frugiperda* does not overwinter in northern Florida, Georgia, and Alabama (Sparks 1979); consequently, any resistant individuals presumably would not survive until the next season.

Pyramiding where multiple genes or events are expressed simultaneously in the same plant has the potential to improve the efficacy of "nonhigh-dose" events, thereby reducing the risk of target pest resistance to Bt toxins. However, cross-resistance to Bt toxins with shared binding sites has been shown in tobacco budworm, *Heliothis virescens* (F.), where a Cry1Ac-resistant strain was also resistant to Cry2A toxin (Gould et al. 1992) and another Cry1Ac-resistant strain was also resistant to Cry1F and other Cry1A toxins (Gould et al. 1995). These results suggest that cross-resistance may pose a risk to strategies where Bt toxins are pyramided in the same plant.

MON84006 singly and pyramided with MON810 had superior control whorl-stage damage by *S. frugiperda* and ear damage by *H. zea* compared with MON810 alone. At this time, MON84006 has not been approved for production. Improved control of whorl and ear infestations by *H. zea* and *S. frugiperda* would increase the flexibility of planting corn and permit double cropping of corn in areas where these pests perennially cause damage.

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